TECHNICAL GUIDELINES

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INTEGRATED GUIDELINES FOR EXTRA VIRGIN OLIVE OIL PRODUCTION CHAIN AND QUALITY CONTROL ASSESSMENT FOR THE FINAL PRODUCT

Requirements

Ente Nazionale di Ricerca e promozione per la standardizzazione - The National Institution of Italy for Standardization Research and Promotion

ENR

This document (Technical Guidelines ENR 10004:2024) was drawn up as part of the ERA HDHL KH FNS SYSTEMIC project: *An integrated approach to the challenge of sustainable food systems: adaptive and mitigatory strategies to address climate change and malnutrition* (Joint EU initiative JPI (A healthy diet for a healthy life), JPI Oceans and FACCE-JPI (JPI Agriculture, Food Security and Climate Change)). This document contains the integrated guidelines for extra virgin olive oil production chain and quality control assessment for the final product.

This document (Technical Guidelines ENR 10004:2024) was drawn up by the Technical Committee composed of the following institutions and organizations: The National Institution of Italy for Standardization Research and Promotion (ENR), University of Palermo (Department of Agricultural, Food and Forestry Sciences), University of Pisa (Department of Agricultural, Food and Agro-environmental Sciences).

After an initial verification of the coverage and completeness of the applicable regulatory framework and identification of any regulatory gaps, ENR drafted this Technical Guidelines based on the experience gained and considering the assessments made in the project.

The ENR standards are revised, when necessary, with the publication of new editions or updates. It is therefore important that users of the same make sure they have the latest edition and any updates.

This standard has been drafted trying to take into consideration the points of view of all interested parties to represent the real state of the art of the matter.

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1. GENERAL PART

The importance of an increasingly marked characterization of quality extra virgin olive oil is highlighted by the needs of a market in which global producers compete.
It is precisely in the awareness that guaranteeing food and nutritional safety is a complex issue that requires an integrated food systems perspective, that the specific and peculiar study of a supply chain, in all its components, such as that relating to the olive growing supply chain, ensures, from on the one hand, a series of results that increase knowledge on a food/condiment as important for the diet as extra virgin olive oil and, on the other, allow us to understand and build a model of knowledge and study to be applied, more in general, to food supply chains.
Building a model and, therefore, drafting a series of guidelines offers the opportunity to create a system more ready to face the social challenges related to food and nutritional security. Food safety is a major challenge for the agri-food sectors. Food security is based on four pillars: availability of food in sufficient quantities; access to food, in a physical and economic sense; use (water quality, nutritional quality, hygiene, distribution of rations, etc.); stability of these three elements over time.

The ISO 22000:2018 standard guarantees safety for all actors in the food supply chain.
The ISO 22000 standard "defines the requirements relating to a food product safety management system whose compliance with the standard can be certified". In practice, it helps supply chain actors to identify and control the risks that threaten food products. Its adoption allows a company to demonstrate that it can guarantee the food safety of the products it supplies or distributes.
The ISO 22000 standard applies to all direct and indirect actors in the food chain, i.e. food producers and food processing companies, transporters, product storage workers, points of sale and suppliers of packaging, ingredients or additives. This standard offers guidance to organizations in developing a food safety management system, which is based on the PDCA (Plan, Do, Check, Act) cycle. The principles on which it is based are: 1) being able to identify and evaluate any dangerous situation for food; 2) be able to take the necessary measures to correct this situation; 3) guarantee the traceability of all links in the food chain.

The distinctive element of the TECHNICAL GUIDELINES ENR 10004:2024 INTEGRATED GUIDELINES FOR EXTRA VIRGIN OLIVE OIL PRODUCTION CHAIN AND QUALITY CONTROL ASSESSMENT FOR THE FINAL PRODUCT is represented by the ability to constitute a model that can be applied to the concept of the supply chain in general, since it concerns the entire chain: from primary production to

transformation, composition-preservation and, finally, to nutritional value (from field to fork); in a context of full sustainability.
The TECHNICAL GUIDELINES ENR 10004:2024 standard was drawn up based on ISO 22000 and is to be considered as a particularization of the same.

1.1 Introduction[1]

Integrated production is an agri-food production system that uses production and defense methods of agricultural production with the aim of reducing the use of synthetic chemical substances to a minimum and optimizing fertilization in compliance with ecological, economic and toxicological. The general objectives that we intend to achieve with the adoption of the integrated production method are:

1. Improvement of health and hygiene safety and production quality
2. Protection of the environment and conservation of animal and plant biodiversity
3. Raising the level of safety and professionalism of agricultural operators.

1.2 Purpose and field of application

The application of the general principles of agronomic techniques goes from cultivation to harvesting of the crops that are intended to be generated with the integrated production method. They integrate the general principles relating to the defense and control of weeds.

1.3 Choice of the growing environment

The evaluation of the pedoclimatic characteristics of the cultivation area is fundamental for the crops involved. The choice of the cultivation environment must be particularly careful in the event of a new introduction of a crop and/or variety.

1.4 Maintenance of the natural agroecosystem

Biodiversity is more present in agricultural systems and contributes to reducing the use of chemical substances (protecting the main organisms useful for the natural containment of adversities), to protecting environmental resources and to respecting the natural agroecosystem. To consolidate and keep the rate of biodiversity high, we recommend the use of techniques and interventions such as the restoration and construction of hedges, dry stone walls, artificial nests, water reservoirs, polyphytic grassing, alternating mowing of the rows, etc., to be adopted in different agro ecosystems.

1 Sicilian Region, Regional regulations for integrated production, agronomic technical standards, 2022.

1.5 Variety choice and multiplication material

To counteract genetic erosion, conserve biodiversity and maintain the typical nature of agricultural areas, the use of local varieties is recommended, considering the varieties that are resistant and/or tolerant to the main plant diseases and the market needs of the products obtainable.

1.6 Preparation of the soil for planting and sowing

The soil preparation work for planting and sowing must be carried out with the aim of protecting and refining the fertility of the soil avoiding erosion and degradation phenomena. These preparations must be defined according to the type of soil, the crops involved, the location, the risks of erosion and the climatic conditions of the area. They must also contribute to maintaining a high biodiversity of the microflora and microfauna of the soil and a decrease in compaction phenomena, allowing the removal of excess rainwater.

1.7 Cultural succession

An agronomically correct crop succession represents a fundamental tool for preserving soil fertility, biodiversity, preventing adversities and safeguarding/improving the quality of production.

1.8 Sowing, transplanting, planting

The sowing and transplanting methodologies for both annual and perennial crops must allow the achievement of adequate productive yields, limiting the adverse impact of weeds, diseases and pests, optimizing the use of nutrients and allowing water savings. These methods, together with other sustainable agronomic practices, have the aim of limiting the use of synthetic plant growth regulators.

1.9 Soil management and agronomical practices for weed control

Soil management and related processing techniques must be aimed at developing crop adaptation conditions to maximize production results, improve nutrient efficiency, keep the soil in good structural conditions, avoid erosion and landslides, maintain the content in organic substance, and favor the penetration of rainwater and irrigation water.

1.10 Tree and fruit management

The treatments intended for tree crops such as pruning, bending, pollination and thinning must be practiced promoting a correct balance of the qualitative and quantitative needs of the production and to improve the health status of the crop.

These management methods must aim to reduce the use of plant growth regulators as much as possible.

1.11 Fertilization

Fertilization aims to improve the level of nutritional elements in the soil and represents the first step towards safeguarding fertility. It must be integrated into a management process for agricultural soils that allows the objective of fertilization to be achieved, i.e. the improvement of the characteristics and qualities of the soil to offer the consumer better agricultural products from a hygienic-sanitary and qualitative point of view.

1.12 Biostimulants and corroborants

The use of corroborants and invigorating products, useful for improving the natural defenses of crops and limiting the appearance of fungal diseases, parasitic insects and physiopathologies caused by cultivation errors, can help perfect the physiological and nutritional state of crops. A crop that is in an optimal physiological-nutritional state is more protected from the attack of physiopathologies and phytopathologies; the advantage of having innovative technical means represents a tool for obtaining greater resistance of crops to biotic and abiotic stresses in integrated defense.

1.13 Irrigation

Irrigation must satisfy the water needs of the crop without exceeding the "field capacity" threshold. Savings, rational use, quantitative and qualitative protection, the use of technical-scientific supports, the use of weather forecasts, are all factors to be taken into consideration to achieve the objective of maximum functionality and water saving, while maintaining excellent quantitative and qualitative levels of agricultural production.

1.14 Collection

The correct collection and delivery methods to storage and processing centers guarantee the maintenance of the best quality characteristics of the products. The ripening indices and quality parameters for harvesting operations can be indicated in the crop sheets according to the species and in reference to the destination of the products. The same sheets can also indicate the maximum times for transfer to the processing and storage center.

2. PRODUCT DATA SHEET - EXTRA VIRGIN OLIVE OIL

2.1 Scope

This product data sheet provides a working tool for obtaining products that comply with the protection of the environment and the protection of consumer health. The guidelines described must be applied in companies producing extra virgin olive oil obtained from the milling of olives produced in compliance with the regulations for the integrated production of olive oil.

2.2 Field of application

The field of application includes the production phases ranging from the cultivation of the olives to the harvesting, to their conservation, to transport and storage, to the production process, to the product analysis and the qualitative evaluation of the extra virgin olive oil.

2.3 Definitions

olives: edible fruit of the olive tree, suitable for milling to obtain extra virgin olive oil.

extra virgin olive oil: olive oil having the characteristics listed in Annex I of Regulation (EEC) 2104/2022.

lot of olives: homogeneous quantity of olives (belonging to a single variety, to varieties of the same quality group or to a set of correctly mixed varieties) produced with integrated production techniques, for which it is possible to guarantee identification and traceability.

lot of oil: homogeneous quantity of oil for which identification and traceability can be guaranteed.

additives: any substance not normally consumed as a food as such and not used as a typical ingredient of foods, regardless of whether it has a nutritional value, which is intentionally added to food products for a technological purpose in the production, transformation, preparation, treatment phases , packaging, transport or storage of foods, can reasonably be expected to become, itself or its derivatives, a component of such foods, directly or indirectly (Council Directive 89/107/EEC).

2.4 Ingredients allowed

The only ingredient (raw material) allowed to produce extra virgin olive oil is made up of olives obtained in compliance with the relevant integrated production regulations.

2.5 Ingredients not allowed

It is forbidden to use other ingredients, adjuvants, additives, substances, components, in any phase of the production process, even as technological aids.

2.6 Growing environment

The olive tree is a perennial, long-lived, evergreen and rustic tree, very resistant to drought and capable of living even in marginal soils. Soils with a clayey texture, sensitive to compaction and poorly drained, and those with high salinity should be avoided. The Sicilian territory, for example, is naturally predisposed for the cultivation of olive trees; in fact, there are numerous internal and coastal areas suitable for cultivation from a climatic and pedological point of view.

2.7 Maintenance of the natural agroecosystem

The presence of uncultivated natural areas (hedges, rows of trees, wooded areas, bodies of water, etc.) is essential to guarantee an important reservoir of useful organisms which represent a source of biodiversity essential for maintaining the stability of the system. It is advisable that this surface is not less than 5% of the used agricultural surface. Correct management of natural spaces is necessary so that they can fully exert their beneficial effects. In the land intended for the development of spontaneous plants, it is necessary to avoid any chemical intervention, soil processing and combustion.

2.8 Variety choice and multiplication material

The cultivation of varieties consisting of or originating from genetically modified organisms (GMOs) is not permitted.
The climatic conditions that characterize, for example, the different olive-growing areas of Sicily influence the adaptation of the different cultivars; the action of these bioclimatic factors has determined a selection work that has taken place over the centuries under the guidance of the farmer. Therefore, when choosing the varieties to plant it is advisable to favor the use of consolidated varieties that strengthen the DOP system. It is preferable to use certified virus-free or virus-controlled material.
The nursery material must comply with the quality standards defined at community and national level, for the genetic-health and agronomic quality

aspects. The materials used (rootstocks, buds and scions) must be purchased from nurserymen accredited by the competent authorities at national and/or regional level.

2.9 Setting and preparation of the soil before planting

The arrangement and preparation of the soil must facilitate the removal of excess rainwater, avoid erosive phenomena, reduce the risks of compaction and maintain fertility. For example, to prepare the soil it is advisable to trench or subsoil to a depth of 60-80 cm, which must be followed by plowing at a depth of no more than 30-40 cm aimed at improving the structure of the soil and to bury the bottom fertilization.

To correctly establish basic fertilization, a chemical-physical analysis of the soil must be carried out before pre-planting work.

2.10 Crop rotation

Before proceeding with replanting, it is advisable to:

1. Leave the soil to rest for at least two years (during which extensive cultivation or green manure can be practiced)
2. Remove root residues from the previous crop
3. Carry out abundant fertilization with organic substance
4. Place the new plants in a different position from that occupied by the previous ones
5. Use the appropriate coupling.

2.11 System and choice of training form

The new plants must be created with spacing that allows, in relation to the fertility of the soil, the possibility of practicing irrigation and the varietal characteristics, good lighting and good ventilation also of the internal parts of the foliage. It is also possible to use intensive spacing, which allows all cultivation operations to be mechanized.

2.12 Breeding and canopy management

Pruning serves to regulate the vegetative and productive activity of the plant and is aimed at ensuring production as constant as possible and maintaining the chosen training method. Biennial pruning, which determines a strong alternation of production, can be replaced, especially in the presence of highly fertile soils, with annual shearing interventions which ensure a slight thinning of the foliage and constant fruiting of the plant. Ordinary practice is green pruning carried out in summer, which consists of eliminating suckers.

2.13 Soil management

Soil management is of fundamental importance for protection from erosion and the conservation of organic substance. Soil management and related processing techniques must be aimed at improving crop adaptation conditions, promoting weed control, improving nutrient efficiency, reducing losses through leaching, runoff and evaporation, keeping the soil in good structural conditions, prevent erosion and landslides, preserve the organic substance content and promote the penetration of rainwater and irrigation water.
Grass cover is very useful on sloping land, to contain soil losses due to surface erosion. The grassing can be carried out with spontaneous herbaceous species or by sowing grass or legume species or with a combination of both.
The excessive number of processes involves a degradation of the organic substance but also a risk of erosion. To reduce this criticality, it is recommended to reduce processing and the use of cover crops to a minimum. In particular, autumn processing is recommended which allows for the burial of cover crop seeds and winter fertilization and subsequently spring chopping followed by burial (of the plant material composed of cover crop biomass and winter pruning residues) to allow the increase in organic substance. To manage green pruning residues and summer weeds, summer shredding is recommended. Through sustainable management it is therefore possible to go from 7 to 3-4 light processes. These strategies aim to reduce the use of non-renewable resources and limit pollution, while at the same time preserving the fertility of the soil, improving its health, structure and its ability to retain essential nutrients for plants. The introduction of cover crops not only protects the soil from erosion, but also helps maintain a balanced and resilient agricultural ecosystem.

2.14 Fertilization

The supply of fertilizing elements must maintain and improve soil fertility, compensate for crop removals and technically unavoidable losses. Fertilization must maintain a nutritional availability in the soil proportional to the needs of the plants in the different phenological phases to maintain the balance between vegetative and productive activity.
The fertilizer inputs can be defined using a model with standard dose sheets which provides, in the presence of a normal production situation, standard quantities of nitrogen, phosphorus and potassium (average values regardless of density and production approximately 20 kg/ha N, 10 kg/ha P and 10 kg/ha K), which may undergo variations depending on production levels. Fertigation distribution of nutrients is preferable. It is advisable to distribute organic matter and fertilizers based on phosphorus and potassium in the autumn/winter period. By distributing fertilizer in the winter period, you risk loss due to run-off and further negative consequences for the environment. This problem can be addressed by monitoring

the content of macro and micronutrients in the soil and the nutritional status of the plant through foliar diagnostics, to provide nutritional supplies in adequate periods and quantities. This practice allows for a reduction in fertilizer requirements of up to 30% and a reduction in management costs in terms of purchase, transport and distribution. The use of pollen, sewage, manure, livestock and agri-food industry waste, compost and products permitted in organic production is permitted, in compliance with current regulations.

2.15 Irrigation

Correct use of the water resource allows the satisfaction of the water needs of the crop and the achievement of economically competitive quantitative and qualitative results, while ensuring the avoidance of waste, the leaching of nutrients and containing the development of adversities. Low volume distribution systems (micro-sprinkling and sub-irrigation) should be preferred, as they allow greater irrigation efficiency to be achieved. The watering volumes and shifts must be evaluated in relation to the cultivation environment, the seasonal trend and the humidity of the portion of soil explored by the roots.
The use of the water resource could be maximized by monitoring the water and phenological status of the plants, to provide the useful quantity for constant and quality production.

2.16 Integrated defense and weed control

In traditional olive growing, phytosanitary treatments are often carried out following a fixed calendar, without adequate monitoring of pathogens. This approach can lead to unnecessary and excessive use of phytochemicals with potential negative consequences for the environment. The frequent and indiscriminate application of these products can contaminate the soil, water and ecosystem, compromising biodiversity and human health. Monitoring allows treatments to be applied only when pathogen levels exceed critical thresholds. The use of chemical products only when strictly necessary and the preference for those with low environmental impact and easily degradable promote more sustainable management of the olive grove. It is essential to implement monitoring systems to continuously evaluate the presence and levels of pathogens in olive groves.

2.17 Collection

The optimal harvest time generally coincides when almost all the drupes are in the early to full veraison phase and the pulp is light or just wine-red in colour. Harvesting can be done manually or by mechanical means. The collection of fruit that has naturally fallen to the ground is prohibited. Furthermore, the use of abscission products is not permitted. The storage phase of the olives, in bins or perforated boxes, must be limited to a minimum (recommended time between harvesting and pressing no more than 24 hours). It is therefore essential to calendarize and plan the harvest to avoid problems such as the olives being left in the mill yards.

2.18 Product transport

Transport must be carried out with suitable means capable of guaranteeing the integrity of the drupes. The types of olives must be recorded in a special sheet containing the weight of the olives and the sampling of the drupes before pressing. The olives must be stored in rigid and ventilated containers, and stored in cool, dry and ventilated rooms.

2.19 Milling

Each milling phase has a significant impact on the final quality of the extra virgin olive oil. Oil extraction can be carried out with a continuous or discontinuous system, i.e. by centrifugation, pressure and percolation. Milling must take place within 24 hours of harvesting. Milling is permitted by maintaining low operating temperatures, through extraction processes such as to maintain the temperature during the processing phase within 27°C. Only the use of drinking water at a temperature such as to maintain the temperature of the olive paste within 27°C and in quantities not exceeding 50% of the weight of the olives is permitted. In the case of a continuous system, it is advisable to adopt extraction systems that reduce or eliminate the use of additional water. Oil production must be consistent with the quantity of registered olives arriving.

3. PRODUCT ANALYSIS AND QUALITATIVE EVALUATION

For the purposes of greater understanding of the product analysis and qualitative evaluation of EVO, a brief representation of the different types of olive oil is referred to. Olive oil is among the most sophisticated foods; the most common frauds are those relating to extra virgin olive oil, which can be marketed as such even if it is not, because refined oils, pomace or seeds have been added. Another case is that relating to the sale of seed oil as olive oil, or of an oil with analytical parameters that do not comply with the product category.
Within the European Community, the classification of olive oils and olive pomace oils is strictly regulated, establishing for each type of limit values to be respected about certain parameters. To verify whether a type of oil falls into a specific product category or whether this batch has undergone adulteration, it is necessary to carry out chemical analyses, according to procedures codified by specific European regulations (EEC Regulation 2022/2104)[2].
The analyzes that are performed on samples of each type of oil for the purposes of their identification from a bio-physical-chemical point of view have the aim of evaluating:

- The quality, determined through the parameter’s acidity, number of peroxides, UV analysis, acid composition, sterol composition, content of halogenated solvents and gas chromatographic analysis
- The shelf life, evaluated through the number of peroxides, UV analysis, acidity, panel tests
- Genuineness, measured by UV analysis, the determination of the acid and sterol composition and the analysis of halogenated solvents.

3.1 Determination of free fatty acids

The acidity of an oil depends on the state of conservation of the raw material and expresses the percentage of free fatty acids contained in it. The fatty acid composition of olive oil is based on the variety, climatic conditions and production site, while the free fatty acids are due to the hydrolysis of triglycerides. The presence of free fatty acids also negatively influences the stability of the product, leading to an amplification of the degradation kinetics. This parameter can be expressed in different ways, depending on the dominant fatty acid in the fatty substance. As a reference, lauric acid is used for coconut oil, palm kernel oil and the like, while palmitic acid is used for palm oils and oleic acid for all other types of oil (including EVO oil). Alternatively, the acid number expresses the mg of KOH necessary to neutralize 1 g of fatty substance.

2 COMMISSION DELEGATED REGULATION (EU) 2022/2104 of 29 July 2022 supplementing Regulation (EU) no. 1308/2013 of the European Parliament and of the Council as regards marketing standards for olive oil and repealing Regulation (EEC)

The COMMISSION DELEGATED REGULATION (EU) 2022/2104 of 29 July 2022 establishes the maximum acidity allowed for extra virgin olive oils (EVO): the acidity is between 0 and 0.8%.
The acidity of an oil sample is determined by titration with KOH (or NaOH) in alcohol/ether, using phenolphthalein as an indicator.
The olive oil samples are dissolved in a mixture of ethanol: ether, in 1:2 and then titrated, until the color change of phenolphthalein (1% ethanol solution) with NaOH according to the method required by EEC Regulation 2022/2104).
The acidity, expressed as a percentage of oleic acid, is equal to:

$$FFA = V \times c \times \frac{M}{1000} \times \frac{100}{m}$$

where:
V = volume of the standard NaOH solution used expressed in milliliters
c = exact concentration in moles per liter of the titrated NaOH solution used
M = molar weight in grams per mole of the acid used to express the result (= 282)
m = weight of the sample in grams.

3.2 Determination of the value of peroxides

Olives damaged during harvesting can activate oxidative processes with high levels of peroxides and therefore a poor ability to preserve the product. The quantity of peroxides is an indicator of the oxidation state of the oil; depends on poor handling and storage.
The limits for the number of peroxides in extra virgin olive oil are indicated in the COMMISSION DELEGATED REGULATION (EU) 2022/2104 of 29 July 2022: Number of peroxides (mEq O_2/kg) ≤ 20.
The value of the peroxides, referring to primary oxidation, is determined with an iodometric titration with a 0.01 N sodium thiosulphate solution, using starch solution, in accordance with the provisions of EEC Regulation. The value of peroxides (PV) is expressed in milliequivalents of active oxygen per kilogram of oil, and is given by the formula:

$$P.V. = \frac{V \times T}{m} \times 100$$

where:
V = volume of mL of standardized sodium thiosulfate solution
T = exact normality of the sodium thiosulfate solution used
m = the weight in g of the sample to be analyzed.

3.3 Determination of spectrophotometric parameters

The spectrophotometric examination of olive oil (Reg. EEC 2104/22) is carried out on a 1% solution of olive oil in isooctane, recording the absorption spectrum in the range between 200 and 300 nm. The specific absorbance values at 232 nm and 270 nm are then calculated.

$$K_\lambda = \frac{A_\lambda}{C \times b}$$

where:
K_λ = specific absorption coefficient at the required λ
A_λ = absorbance at wavelength λ
C = concentration in g/100 mL
b = cell thickness in cm

The ΔK referring to the height of any peak located at 270 nm is also calculated

$$\Delta K = K_m - \frac{K_{m-4} + K_{m+4}}{2}$$

where:
Km = absorption coefficient at the peak at 270 nm (m)

EU regulation no. 2104/2022 established the following limits for extra virgin olive oil:
$K_{232} \leq 2{,}50$; $K_{270} \leq 0{,}22$; $\Delta K \leq 0{,}01$.

3.4 Preparation of phenolic extracts and determination of the phenolic content

For the preparation of phenolic extracts from EVO, it is possible to use the method described by Montedoro et al. (1992) with some changes:

- - carry out liquid/liquid extraction (LLE) on the oil samples with a solution of methanol: water (80:20 v/v)
- - mix 10 mL of oil samples with 10 mL of the mixing solution
- - shake the mixture vigorously for 3 minutes and let it rest in the dark for 3 minutes, and finally centrifuge for 15 minutes at 2683 rpm to separate the two phases
- - repeat the extraction two more times
- - store the supernatant solutions obtained overnight at -20 °C in an N_2 atmosphere, until they are used for the determination of the total phenol content.

The total phenolic content of olive oil samples can be determined colorimetrically at 765 nm, using the Folin-Ciocalteau reagent. This method is based on the chemical oxidation of phenolic compounds by an oxidizing mixture, called Folin-

Ciocalteau reagent, made up of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) which, upon reduction, forms a mixture of blue-colored oxides, the intensity of which is directly proportional to the number of phenolic residues present on the antioxidant molecule. The results of the absorbances obtained are expressed as mg of gallic acid equivalents of sample. The total phenolic content is determined using a gallic acid calibration curve as a standard and applying the Lambert-Beer law.

3.5 Determination of antioxidant power

The ABTS assay can be used to evaluate the antioxidant power of EVO. This spectrophotometric test allows the antioxidant power of olive oil to be determined through the reaction between the sample to be analyzed with a radical cation. In this assay, the colored and stable cation radical (R^+) is generated by oxidation of the diammonium salt of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by means of a potassium persulfate solution ($K_2S_2O_8$). The radical cation thus generated is very stable over time and is characterized by an intense blue-green color (with absorption maxima located at 415, 645, 734 and 815 nm). This radical cation ($ABTS^+$) has an absorption peak at 734 nm. Following the addition of one or more antioxidants (AOH) in the reaction environment, these release one or more hydrogen atoms to the cationic radical, thus determining a decrease in the initial absorbance value, causing a discoloration of the solution. The decrease in the peak at 734 nm of the radical cation ($ABTS^+$) after a pre-established incubation time is then analyzed under UV-Vis. This decrease (discoloration) is proportional to the antioxidant charge present in the sample.
The antioxidant capacity will be determined by calculating the percentage of inhibition, referred to a reference standard, with the formula:

$$\%A_{734} = (1 - A_c \div A_0) \times 100$$

where:
A_c = sample absorbance
A_0 = radical absorbance

The final value is compared with a standard of Trolox, analogue of vitamin E, expressing the unit of antioxidant activity as TEAC (Trolox Equivalent Antioxidant Capacity). The antioxidant activity of the samples was quantified through a Trolox dose-response curve, constructed in the concentration range 0.2-1.5 expressed as $TEAC/g^{-1}$.

3.6 Organoleptic analysis of evo oil

Organoleptic analysis has a significant importance in the qualitative evaluation of an oil. To recognize an oil as extra virgin it is necessary, in fact, not only to ascertain its chemical characteristics but also its sensorial quality. In the EEC Reg. n. 2104/22 it is established that, to define the product category to which it belongs, an oil must be subjected to tasting by a group of selected tasters (Panel Test), who must evaluate its color, flavor and smell and measure its intensity. The Panel Test is made up on average of 10 tasters, each of whom carries out the organoleptic analysis separately, indicating on a specific sheet the presence and intensity of the basic sensations (defects and merits codified by the Regulation). The positive descriptors used in the sensorial evaluation of extra virgin olive oils are exclusively olive fruitiness, bitterness and spiciness (the method's main objective is to ascertain the absence of defects for the purposes of product classification). The measurements are then processed, and the medians are plotted on a star diagram. The classification of EVO oil is defined based on the median of the most perceived defect, both in terms of intensity and frequency.

3.7 Determination of the intensity of bitterness

The intensity of bitterness, correlated to the presence of oleuropein in olive oil, can be determined using the Gutiérrez et al. method (1992). Bitter components are extracted from 1.0 ± 0.01 g EVO samples using disposable sepack (C18) extraction columns (J.T. Baker Chemical Company, Phillipsburg, NJ, USA). The absorbance was recorded at 225 nm. The intensity of the bitterness is therefore given by the following formula:

$$BI = 13.33 \times K_{225} - 0.837$$

where:
K225: assorbanza della soluzione alla lunghezza d'onda di 225 nm

4 STORAGE, PRESERVATION AND PACKAGING OF OIL

The oil must be stored in suitable rooms; thermally insulated, dry and protected from sunlight. The containers used for storage must be carefully clean and made of inert material (stainless steel or tinted colored glass) to protect the product from light and such as to reduce the surface in contact with the air as much as possible. To maintain the original organoleptic characteristics of the oil, it is recommended to store the product under nitrogen.

Extra virgin olive oil must be marketed in containers, in accordance with the law. The use of containers that reduce the risk of photo-oxidation and heat exchange is preferable. We recommend the use of inert gases in the bottling phase and suitable hermetic closure systems, impervious to oxygen.